

Tissue processing for small capillary immunohistochemistry

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 An abbreviated version of this protocol was published in eLIFE in Nov 2019

Endothelial EphB4 maintains vascular integrity and transport function in adult heart

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Detailed protocol

Tissue processing for small capillary immunochemistry

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Material and Reagents

- Dissecting tools
- PFA (Roth, 0335.3)
- PBS (Gibco, 18912-014)
- Sucrose (Sigma, S0389)
- Gelatin from porcine skin (Sigma, G1890)
- Polyvinylpyrrolidone (Sigma, P5288)
- EDTA (Invitrogen, 15575-038)
- Disposable Base Moulds (Thermo Scientific, 58950)

Equipment

- Cryostat Leica CM3050 S

Procedure for bone and liver samples

1. Tissue is dissected in cold PBS and fixed overnight in 4% PFA in PBS at 4°C with rocking.
2. Wash with PBS, 3 times for 10 minutes at 4°C with rocking.
3. Cryo-Protection with a series of consecutive overnight washes in PBS containing increasing concentration of sucrose. PBS / 10% Sucrose, overnight at 4°C with rocking.
4. PBS / 20% Sucrose overnight at 4°C with rocking.
5. PBS / 30% Sucrose overnight at 4°C with rocking.
6. Tissue was embedded in a embedding solution (15% sucrose, 8% gelatin, and 1% polyvinylpyrrolidone in PBS). Base moulds are filled with embedding solution and samples are then immersed in the solution. After waiting for the embedding solution to solidify, approximate 20 minutes, samples are frozen at -80°C.
7. Samples are sectioned 50 µm thick in the cryostat and collected into microscopy slides. After sectioning, samples were let dry overnight at room temperature and stored at -20°C.

Procedure for bone samples

1. Bones are dissected in cold PBS and fixed overnight in 2% PFA in PBS at 4°C with rocking.
2. Wash with PBS, 3 times for 10 minutes at 4°C with rocking.
3. Decalcification with two consecutive overnight 0.5M pH 8.0 EDTA washes at 4°C with rocking.
4. Cryo-Protection with a single wash of 20% sucrose and 1% polyvinylpyrrolidone in PBS, and finally embed as described above.

How to cite: Readers should cite both the Bio-protocol article and the original research article where this protocol was used:

1. Luxán G. (2020). Tissue processing for small capillary immunochemistry. Bio-protocol in prep.
2. Luxán G, Stewen J, Díaz N, Kato K, Maney SK, Aravamudhan A, Berkenfeld F, Nagelmann N, Drexler HCA, Zeuschner D, Faber C, Schillers H, Hermann Wiseman J, Vaquerizas JM, Pitulescu ME, Adams RH. (2019) Endothelial EphB4 maintains vascular integrity and transport function in adult heart eLife 2019;8:e45863 doi: 10.7554/eLife.45863

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1. Luxán, G. , Pitulescu, M. and Adams, R. (2020). Tissue processing for small capillary immunochemistry. Bio-protocol Preprint. [bio-protocol.org/prep212](https://doi.org/10.21203/rs.3.rs-1000000/v1).
2. Luxán, G., Stewen, J., Díaz, N., Kato, K., Maney, S. K., Aravamudhan, A., Berkenfeld, F., Nagelmann, N., Drexler, H. C., Zeuschner, D., Faber, C., Schillers, H., Hermann, S., Wiseman, J., Vaquerizas, J. M., Pitulescu, M. E. and Adams, R. H. (2019). Endothelial EphB4 maintains vascular integrity and transport function in adult heart. eLIFE. DOI: [10.7554/eLife.45863](https://doi.org/10.7554/eLife.45863)

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